

# Some Aspects of DEHP and its Action on Lipid Metabolism

by Michael S. Stein,\* Priscilla I. Caasi,\* and Padmanabhan P. Nair\*†

In 1967, Nazir et al. (1,2) reported the specific localization of di-2-ethylhexyl phthalate (DEHP) in bovine heart muscle mitochondria. This led us to investigate the action of dietary DEHP in a number of selected tissues.

Preliminary, unpublished studies have indicated that adipose tissue and total body weights were affected by the addition of DEHP to the diet. The purpose of this study was to determine whether oral DEHP administration affects lipid or DEHP concentrations in a select number of organs. Another objective of this work was to determine whether tissue DEHP and lipid levels were affected by removal of fat from the normal diet. The heart, liver, and epididymal fat pad were used for experimental determinations.

## Experimental

Thirty-six male, weanling, albino rats of the Wistar strain were divided into four groups. The animals were arranged such that the mean body weight per group was equal.

Two basal diets were prepared: diet I, which contained 4% fat, and diet III, which was fat-free. The experimental diets II and IV were supplemented with 0.1% DEHP.

Table 1 shows the main dietary constituents, which were obtained from Nutritional Biochemicals Corporation. The caloric

content of each of the four diets is approximately equal.

Extreme care was taken to avoid the use of plastic products, such as spoons and wash bottles.

Forty-four day paired-feeding was accomplished by permitting the animals to eat *ad libitum*, in which the upper food intake limit was established by animals in Group I. The mean daily food intake per rat was  $14.4 \pm 0.6$  g (one standard deviation).

After six weeks, the rats were sacrificed by exsanguination from the abdominal aorta, under ether anesthesia. The heart, liver, and epididymal fat pad were homogenized in chloroform-methanol (2:1). The homogenate was filtered and centrifuged to yield a supernatant, free of tissue traces. In order to determine lipid concentration, aliquots of supernatant were evaporated, and the lipid percentage was calculated.

## Determination of DEHP in Tissues

DEHP was quantitatively determined by a combination of preparative thin-layer chromatography and analytical gas-liquid chromatography. An aliquot of the supernatant equivalent to 0.1–0.5 g of tissue was evaporated under nitrogen. Two 1-ml aliquots of 4% ether in hexane were used to extract DEHP. This solution was then evaporated in preparation for thin-layer chromatography.

Thin-layer chromatography was performed on silica gel G, a mixture of 1,2-

\*Biochemistry Research Division, Department of Medicine, Sinai Hospital of Baltimore, Inc., Baltimore, Maryland 21215.

†To whom reprint requests should be sent.

Table 1.  
Composition of diets in different experimental groups.

Dietary components	Composition, %			
	Group I	Group II	Group III	Group IV
Sucrose	70	69.9	74	73.9
Vitamin-free casein	20	20	20	20
Lard	4	4	—	—
Salt mixture	4	4	4	4
Vitamin mixture	2	2	2	2
DEHP	—	0.1	—	0.1
Calories per 100 g	397	397	377	377

dichloroethane and benzene (1:1) being used as the mobile phase. Each plate was divided into five channels. A 50  $\mu$ g standard in the first channel was detected with Rhodamine 6 G under ultraviolet light, and this served as a marker to locate the DEHP zone. The second channel served as a control for the residues from the solvents without any biological material. During the final calculations, the reagent blank values were subtracted from the experimental values in order to eliminate contamination from the environment. The other three channels were used for the biological samples, which were plated using 4% ether in hexane. The approximate development time was  $\frac{1}{2}$  hr, and the average  $R_f$  value was 0.47.

The zones corresponding to DEHP were scraped and the silica gel was extracted with two 5-ml aliquots of ether and evaporated under nitrogen. The samples were then injected into the gas-liquid chromatograph with the use of hexane as a solvent. A standard 6 ft x  $\frac{1}{4}$  in. glass column packed with 80-100 mesh Gas-Chrom P coated with a mixture of S E 52/X E 60 was used (3). A Barber-Colman Model 10 gas chromatograph equipped with a  $^{90}\text{Sr}$  argon ionization detector was used in all these determinations. A column temperature of 205°C and 20 psi yielded the peak in 6 min. The peak areas were triangulated and compared with the standard. The minimum percentage recovery by use of this technique was 80%.

## Analysis of Data

Analysis of variance, first-order interaction was employed to analyze the data. The results are expressed as means and their standard error of mean.

## Results and Discussion

Figure 1 shows the mean liver lipid concentration  $\pm$  the standard error of the mean for each of the four groups. The values are expressed as milligrams lipid per gram liver. A comparison of the results obtained for diets I and III, indicates fat deprivation caused an increase in hepatic lipid deposition. The addition of 0.1% DEHP to the normal fat diet caused an increase in the deposition of hepatic lipid (diet I vs. diet II). On the other hand, addition of the same amount of DEHP to fat-free diet counteracted the effect of fat deprivation in the diet.

The total liver lipid (Fig. 2) shows that there is a significant increase in lipid deposition with dietary DEHP in animals fed the 4% fat diet (diet I vs. diet II). Moreover, animals on a fat-free diet (diet III) had a

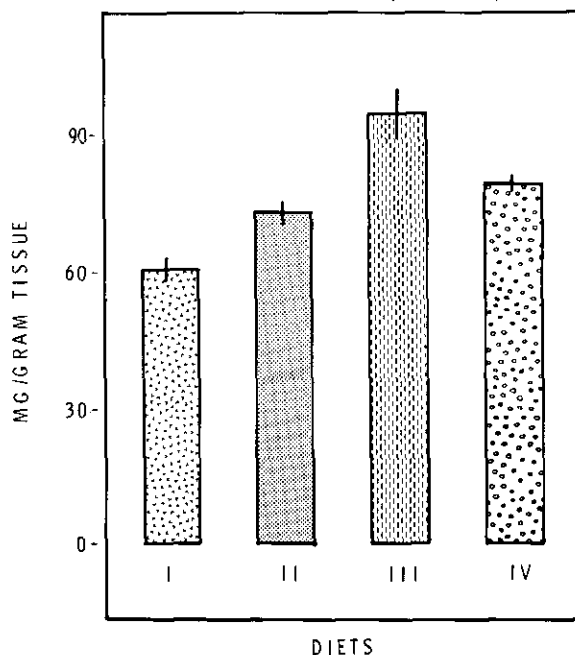


FIGURE 1. Total lipid concentration in liver. Values are expressed as mg/g liver, wet weight.

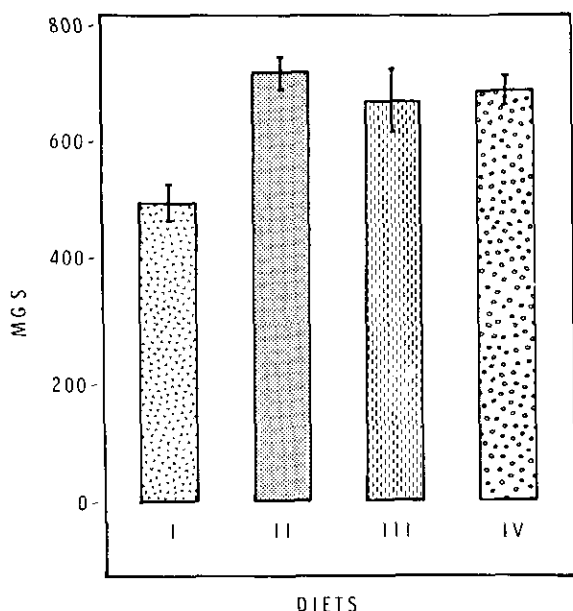


FIGURE 2. Total lipid concentrations in liver. Values are expressed as mg/g liver, wet weight.

greater lipid content than those fed a fat-supplemented diet (diet I).

Figure 3 represents data on the mean liver weights of animals in each experimental group. Addition of DEHP to either fat-free or normal diets resulted in a significant hypertrophy of the liver. We have also observed that addition of DEHP to these diets did not result in an accumulation of DEHP in the liver (graph not shown).

Figure 4 shows that the total lipid content of the heart remained constant regardless of dietary fat or dietary DEHP.

In contrast to the liver, supplementation of the diets with DEHP resulted in a significant deposition of DEHP in the heart in both dietary groups (Fig. 5).

Similarly, Figure 6 shows that DEHP accumulates in the epididymal fat pad of animals fed DEHP. Figure 7 shows that dietary DEHP has a significant influence on the total weight of the epididymal fat pad in the following manner. Animals on the 4% fat diet showed an increase in fat pad weight, while those on the fat-free diet showed a decrease upon addition of DEHP to the respective diets. In other words, addition of DEHP to these diets resulted in an equal but opposite effect on this tissue.

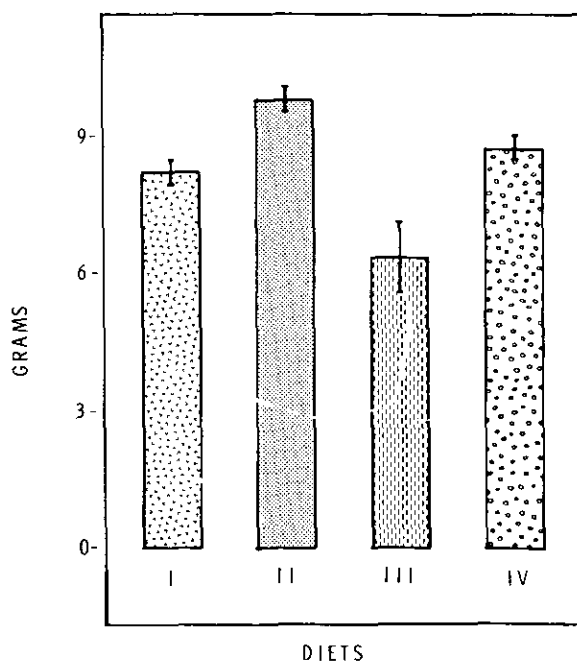


FIGURE 3. Mean liver weight in each experimental group.

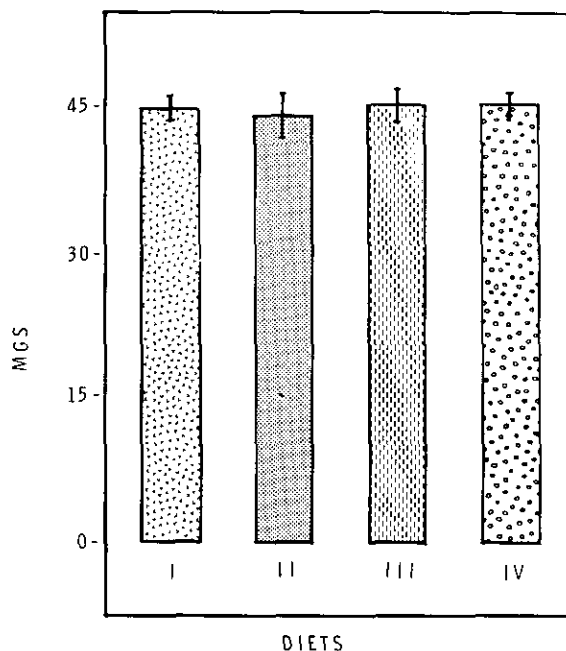


FIGURE 4. Total lipid concentrations in heart. Values are expressed as mg/g heart, wet weight.

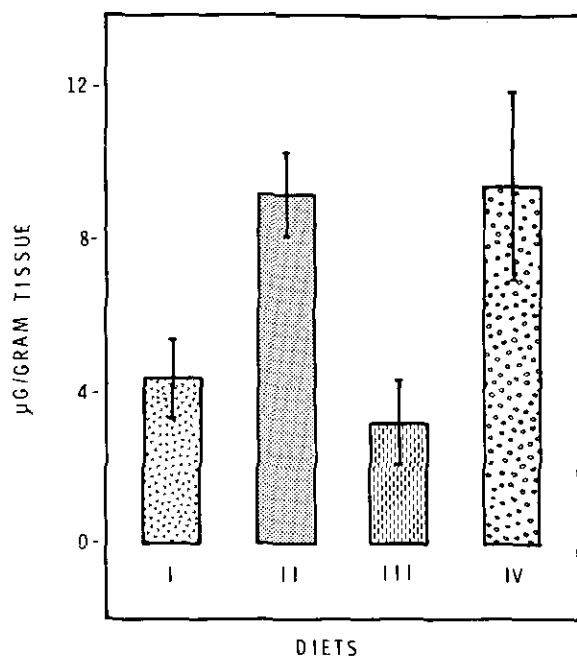


FIGURE 5. Total DEHP concentration in heart. Values are expressed as  $\mu\text{g/g}$  heart, wet weight.

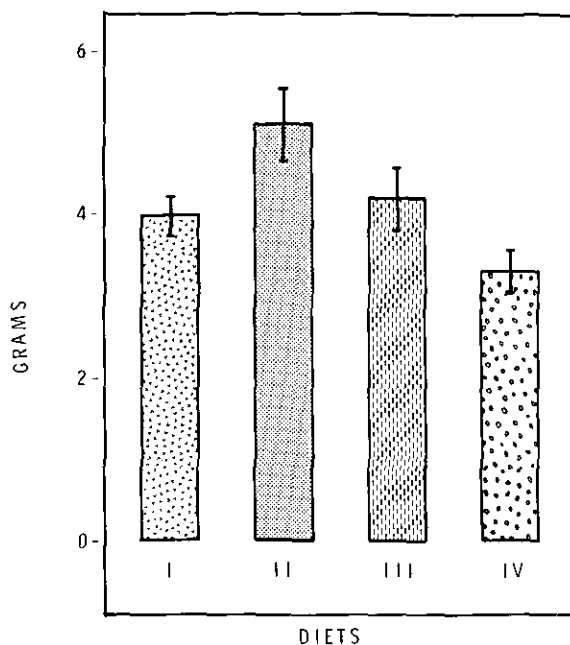


FIGURE 7. Mean epididymal fat pad weight in each experimental group.

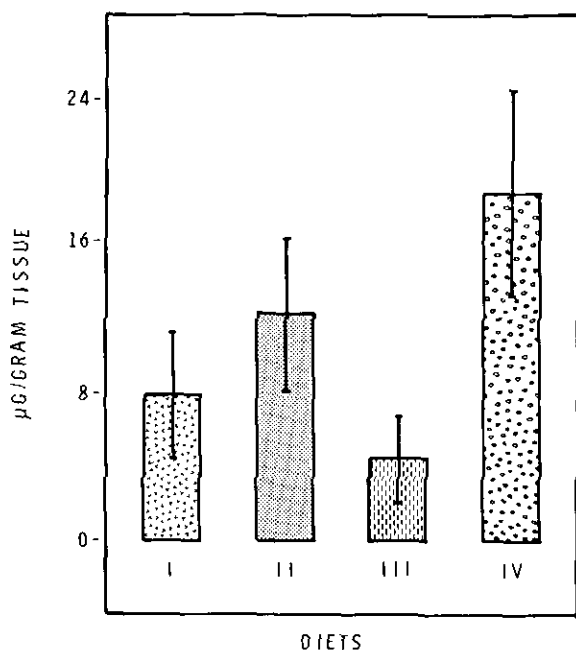


FIGURE 6. Total DEHP concentrations in epididymal fat pad. Values are expressed as  $\mu\text{g/g}$  fat pad weight.

Lastly, a statistical analysis of the mean body weights revealed a 5% increase in the experimental group receiving both fat and DEHP.

#### REFERENCES

1. Nazir, D. J., Beroza, M., and Nair, P.P. 1967. Isolation and identification of di(2-ethylhexyl) phthalate—a component of heart muscle mitochondria. *Fed. Proc.* 26: 412.
2. Nazir, D. J., et al. 1971. Isolation, identification, and specific localization of di-2-ethylhexyl phthalate in bovine heart muscle mitochondria. *Biochemistry* 10: 4228.
3. Nair, P.P., and Luna, Z. 1968. Identification of  $\alpha$ -Tocopherol from tissues by combined gas-liquid chromatography, mass spectrometry and infrared spectroscopy. *Arch. Biochem. Biophys.* 127: 413.